(a) In the edible tissues and in eggs of chickens and turkeys:

(1) 1 part per million in uncooked liver and kidney.

(2) 0.5 part per million in uncooked muscle tissue.

(3) In eggs:

(i) 8 parts per million in egg yolks.

(ii) 4 parts per million in whole eggs.

(b) In the edible tissues of calves:

(1) 2.0 parts per million in uncooked fat.

(2) 0.5 part per million in uncooked muscle tissue, liver, and kidney.

(c) In the edible tissues of pheasants:

(1) 1 part per million in uncooked liver.

(2) 0.5 part per million in uncooked muscle.

[40 FR 13942, Mar. 27, 1975, as amended at 50 FR 18472, May 1, 1985]

§ 556.52 Apramycin.

Tolerances of 0.1 part per million are established for total residues of apramycin in uncooked swine muscle, 0.3 part per million for liver, and 0.4 part per million for kidney and fat. A drug residue assay measuring parent apramycin (the marker residue) in the target tissue, kidney, serves to monitor the total residue in edible tissues. A marker residue concentration of 0.1 part per million in kidney corresponds to 0.4 part per million total residue in this target tissue.

[47 FR l5771, Apr. 13, 1982]

§ 556.60 Arsenic.

Tolerances for total residues of combined arsenic (calculated as As) in food are established as follows:

(a) In edible tissues and in eggs of chickens and turkeys:

(1) 0.5 part per million in uncooked muscle tissue.

(2) 2 parts per million in uncooked edible by-products.

(3) 0.5 part per million in eggs.

(b) In edible tissues of swine:

(1) 2 parts per million in uncooked liver and kidney.

(2) 0.5 part per million in uncooked muscle tissue and by–products other than liver and kidney.

§ 556.70 Bacitracin.

Tolerances for residues of bacitracin from zinc bacitracin or bacitracin methylene disalicylate are established at 0.5 part per million (0.02 unit per gram), negligible residue, in uncooked edible tissues of cattle, swine, chickens, turkeys, pheasants, and quail, and in milk and eggs.

[42 FR 18614, Apr. 8, 1977]

§ 556.90 Buquinolate.

Tolerances are established for residues of buquinolate as follows:

(a) In edible tissues of chickens:

(1) 0.4 part per million in uncooked liver, kidney, and skin with fat.

(2) 0.1 part per million in uncooked muscle.

(b) In eggs:

(1) 0.5 part per million in uncooked yolk.

(2) 0.2 part per million in uncooked whole eggs.

§ 556.100 Carbadox.

No residues of carbadox (Methyl 3-(2-quinoxalinylmethylene) carbazate-N^1, N^4-dioxide) and its metabolite (quinoxaline-2-carboxylic acid) are found in the uncooked edible tissues of swine as determined by the following method of analysis:

I. REAGENTS

A. Benzene—Distilled-in-Glass grade, Burdick and Jackson Laboratories or equivalent.

B. Ethyl acetate—Distilled-in-Glass grade, Burdick and Jackson Laboratories or equivalent.

C. n-Hexane—Distilled-in-Glass grade, Burdick and Jackson Laboratories or equivalent.

D. 1-Propanol—reagent grade, dried over molecular sieve pellets (5A).

E. Citric acid monohydrate—U.S.P., Pfizer, Inc., or equivalent.

F. Potassium hydroxide—pellets, reagent grade.

G. Sodium hydroxide—pellets, reagent grade.

H. Hydrochloric acid—reagent, A.C.S.

I. Sulfuric acid—reagent, A.C.S.

J. Sodium sulfate—anhydrous, reagent grade.

K. Quinoxaline-2-carboxylic acid—Pfizer, Inc., or equivalent.

L. Propyl quinoxaline-2-carboxylate—Pfizer, Inc., or equivalent.

M. Acridine—practical grade; Matheson Coleman and Bell or equivalent.

II. SOLUTIONS

A. 1*M* Citric acid.

B. 5*M* Sodium hydroxide.

C. 3*M* Potassium hydroxide.

D. 0.5*M* Citric acid buffer. Adjust the pH of 100 milliliters of 1*M* citric acid to pH 6.0 with 5*M* sodium hydroxide (approximately 55 milliliters), using a previously calibrated pH meter. Adjust the final volume to 200 milliliters with distilled water. Before making the final pH adjustment, cool the buffer to room temperature.

E. 1-Propanol-sulfuric acid reagent (97:3). Dilute 3 milliliters of concentrated sulfuric acid to 100 milliliters with dried, filtered, and cooled 1-propanol.

F. Acridine solution. Dissolve 1 milligram of acridine in 100 milliliters of benzene.

G. Quinoxaline-2-carboxylic acid solutions:

1. *Stock solution A.* Dissolve 1.25 milligram of quinoxaline-2-carboxylic acid in enough 1-propanol to make 100.0 milliliters (concentration 12.5 micrograms per milliliter).

2. *Stock solution B.* Dilute 1.0 milliliter of stock solution A to 100.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 0.125 microgram per milliliter).

3. *Working standard solution C.* Dilute a 2.0 milliliter aliquot of stock solution B to 10.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 25.0 nanograms per milliliter).

4. *Working standard solution D.* Dilute a 3.0 milliliter aliquot of stock solution B to 10.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 37.5 nanograms per milliliter).

5. *Working standard solution E.* Dilute a 4.0 milliliter aliquot of stock solution B to 10.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 50.0 nanograms per milliliter).

6. *Fortification solution.* Dilute 3.0 milliliters stock solution A to 250 milliliters with distilled water (concentration 150 nanograms per milliliter).

7. *Propyl quinoxaline-2-carboxylate solution.* Dissolve 1.00 milligram of propyl quinoxaline-2-carboxylate in enough ethyl acetate to make 10 milliliters (concentration 100 micrograms per milliliter).

III. APPARATUS

A. Column, glass-tapered at one end, 0.9 centimeters x 21.5 centimeters, prepared from a 10-milliliter serological pipette.

B. Centrifuge tubes, heavy duty—50-milliliter graduated (60-milliliter capacity), equipped with glass stoppers, R. C. Ewald, Inc., or equivalent.

C. Centrifuge tubes—50-milliliter graduated, equipped with glass stoppers.

D. Volumetric flasks—5 10 100 and 250-milliliter capacity, glass stoppered.

E. Pipettes, automatic transfer—10 15 and 25-milliliter delivery volume.

F. Pipettes, measuring—0.1 and 0.5 milliliter delivery volume.

G. Pipettes, volumetric—1 2 3 4 and 5-milliliter delivery volume.

H. Pipette, serological—10 milliliter delivery volume.

I. Pipettes—Pasteur, disposable.

J. Propipette bulb.

K. Syringe—10 microliter capacity, Hamilton or equivalent.

L. Crystallizing dish—190 millimeter (diameter) x 100 millimeter (height), for oil bath.

M. Test tube rack.

N. Test tube mixer—Vortex mixer or equivalent.

O. Lab jack—Cenco or equivalent.

P. Thermo-stir hotplate.

Q. Magnetic stirrer bar (teflon).

R. Thermometer—centigrade, 0° to 150° C. range.

S. Knife (for cutting frozen tissue).

T. Ultraviolet light—254 nanometers and 366 nanometers.

U. Scalpel.

V. Torsion balance—style RX–1, class A, Torsion Balance Co., or equivalent.

W. Cahn electrobalance—Cahn Model C–2 or equivalent.

X. Centrifuge—International, size 2, model K, or equivalent.

Y. Rotary evaporator equipped either with a water aspirator or with a vacuum pump and condenser.

Z. Alkacid test paper.

AA. Glassine paper.

BB. Glasswool.

CC. Flask—round bottom, 29/42 ST, 250 milliliters.

DD. Flask—round bottom, 19/22 ST, 65 milliliters.

EE. Funnel—burette.

FF. Hair dryer.

GG. pH meter.

HH. Tray—instrument, stainless steel.

II. Water bath.

JJ. Precoated thin layer plates—20 x 20 centimeters; 250 micron thickness, Silica gel GF, E. Merck, Darmstadt; distributed by Brinkmann Instruments Inc., Westbury, NY 11590 or equivalent.

KK. Desaga multiplate developing tanks for five 20 x 20 centimeters plates-distributed by Brinkmann Instruments Inc., or equivalent.

LL. Gas-liquid chromatograph—Micro Tek 220 model instrument (or equivalent) equipped with a Ni^{63} electron affinity pulsed detector and a 0–1 MV recorder. Conditions and operating parameters for the gas-liquid chromatograph are: Isothermal column temperature, 175° C.; inlet heater, 270° C.; EC detector temperature, 275° C.; argonmethane

(95:5) flowrate, 100 milliliters per minute (40 pounds per square inch); chart speed, ½ inch per minute, attentuation, 10 x 64. Electrometer pulse parameters: RF mode; voltage output, 55; pulse rate, 270 microseconds; pulse width, 3.0 microseconds.

A glass sleeve injection port liner is installed for off-column injections.

MM. Packing—3 percent OV–17 on Gas Chrom Q, 60–80 mesh, Applied Sciences Laboratories, Inc. or equivalent.

NN. Column—pyrex glass, U-tube, 6 feet (length) x 4 millimeters (inside diameter). Condition the packed column at 280° C. for at least 72 hours with argon-methane (95:5) flow, detached from the detector input.

OO. Septum—high temperature type (HT–13), Applied Sciences Laboratories, Inc. or equivalent.

PP. Detector-Nickel[63] electron capture. The voltage current profile for this detector should plateau at 30 volts or less in the DC mode when a stream of nitrogen gas is passed through the column and the electron capture detector.

IV. PROCEDURE

A. DISSOLUTION AND HYDROLYSIS STEP

Transfer 5 grams of swine tissue (freshly sliced from frozen tissue) to a 50-milliliter centrifuge tube. Add 10 milliliters of 3*M* potassium hydroxide, stopper, and place in a 100° C. silicone oil bath for 1 hour.

NOTE: The level of the silicone oil bath should exceed that of the tissue sample. Stopper the tubes lightly in order to allow the digestion mixture to ''breath''. To determine the recovery of quinoxaline-2-carboxylic acid in swine tissue at the 30 p.p.b. level, fortify 5 grams of sample with 1 milliliter of fortification solution (concentration 150 nanograms per milliliter).

B. EXTRACTION STEP

1. Cool the alkaline hydrolyzate in an ice bath and acidify to 1 pH 1 (deep red to alkacid test paper) with 4 milliliters of concentrated hydrochloric acid. Add 15 milliliters of ethyl acetate to the acidified hydrolyzate, stopper, and extract by shaking for 20 seconds. Centrifuge the mixture at 1,500 revolutions per minute for 5 minutes to clarify the ethyl acetate phase. Recover the ethyl acetate phase with a blowout pipette equipped with a propipette bulb, and transfer this extract to a 60-milliliter separatory funnel equipped with teflon stopcocks. Reextract the hydrolyzate with two additional 15-milliliter portions of ethyl acetate, and combine the organic extracts.

NOTE: Do not contaminate the ethyl acetate phase with interfacial material during these extractions. Quinoxaline-2-carboxylic acid is unstable in strongly acidic solutions. Continue to process these extracts through the benzene extraction and evaporation steps.

2. Add 5 milliliters of 0.5*M* citric acid buffer (pH 6.0) to the ethyl acetate extract, shake, and allow the lower phase to clarify for about 20 minutes. Collect the aqueous phase in a 50-milliliter glass-stoppered centrifuge tube. Reextract the ethyl acetate phase with an additional 5 milliliters of pH 6 buffer, wait for the aqueous phase to clarify, and combine the aqueous extracts. Acidify (pH 1) the aqueous extract with 2 milliliters of concentrated hydrochloric acid, stopper, and extract with 25 milliliters of benzene. Centrifuge to clarify the benzene layer and transfer the organic phase, using a blowout pipette equipped with a propipette bulb, to a 250-milliliter round bottom flask. Repeat the extraction and centrifugation steps three times. Combine the benzene extracts (about 100 milliliters) and evaporate to near-dryness, using a rotary evaporator equipped with a water aspirator and with a water bath set at 40° C.

NOTE: A rotary evaporator equipped with a vacuum pump and condenser may be used at this point. These residues may be stored overnight.

C. ESTERIFICATION STEP

Reconstitute the residue from the previous step by rinsing the walls of the round bottom flask with 2 x 2 milliliters of 1-propanol-sulfuric acid reagent; transfer each rinse with a disposable pipette to a 50-milliliter centrifuge tube. Stopper and heat the tube in a silicone oil bath at 90° C. for 1 hour. Cool the reaction mixture in an ice bath before proceeding to the following extraction step.

NOTE: Samples and standards may be stored overnight at room temperature in the propanol-sulfuric acid medium.

D. EXTRACTION OF THE ESTER DERIVATIVE

Add 10 milliliters of water and 15 milliliters of n-hexane to the esterification mixture. Extract and centrifuge to clarify the n-hexane phase. Transfer the n-hexane extract to a 65-milliliter round bottom flask; reextract the aqueous-propanol phase with two additional 15-milliliter portions of n-hexane. Centrifuge after each extraction and combine the n-hexane extracts. (NOTE: Avoid taking any of the aqueous phase in this extraction step; otherwise, the n-hexane extracts will have to be washed with 3 x 10 milliliters of water and dried over sodium sulfate.) Concentrate this solution to 0.5 milliliter, using a rotary evaporator equipped with a water aspirator and with a water bath set at 25° C. (NOTE: A rotary evaporator equipped with a vacuum pump and condenser may be used at this point.) Fortify this solution with 0.1 milliliter of acridine marker (1 milligram per 100 milliliters benzene).

NOTE: Do not store the n-hexane extracts of the propyl ester derivative overnight. Continue to process these solutions by the following thin-layer chromatography step E.

E. THIN-LAYER CHROMATOGRAPHY

1. Quantitatively transfer the concentrated n-hexane extract to the ''origin'' of a 20-centimeters x 20-centimeters silica gel thin-layer plate, using a disposable pipette. When pipetting this extract, streak it in a uniform band approximately 15 centimeters across and approximately 20 millimeters above the lower edge of the plate, making sure not to scratch or remove appreciable portions of adsorbent and avoiding application of the sample to the sides of the plate. The applied band should not diffuse or penetrate to the end of the silica gel layer, but should remain 10 millimeters above the lower edge of the silica gel layer. Rinse the round bottom flask (containing residual n-hexane) with three portions of approximately 0.25 milliliter each of ethyl acetate; transfer each portion with the same pipette and cover the same area of the plate as described above. Following each application of the extract and ethyl acetate washes, evaporate the solvent from the plate by directing a stream of cool air to the sample zone (''origin''). Prior to chromatographic development, place an edge (approximately 5 millimeters deep) of the thin-layer plate into a tray of ethyl acetate so that the solvent will rise through the applied sample zone to form it into a narrow band approximately 10 millimeters above the ''origin.'' Air dry this plate before chromatographic development.

2. Place the prepared plate in a chromatographic chamber lined with blotting paper and saturated with the benzene-ethyl acetate system (85:15). Develop the plate twice in this system, maintaining straight solvent fronts and allowing the solvent front to reach the top of the plate during each irrigation. Air dry the thin-layer plate for approximately 5 minutes between the first and second irrigations. Each irrigation takes approximately 75 minutes. Developed plates should not be stored overnight. Examine the developed plate under long wavelength (366 nanometers) ultraviolet light and locate the blue fluorescent band of acridine (R_f approximately 0.5). Mark out a 12-millimeters x 20-centimeters band of silica gel encompassing an area 5 millimeters above and 7 millimeters below the center of the acridine marker and extending from one side of the plate to the other.

NOTE: The relative mobilities of propyl quinoxaline-2-carboxylate and acridine must be checked in each laboratory to determine where a 12-milliliter x 20-centimeters zone of silica gel is to be excised in order to quantitatively recover the propyl ester derivative. This may be accomplished by mixing 0.1 milliliter of acridine solution (1 milligram per 100 milliliters) with 0.4 milliliter of propyl quinoxaline-2-carboxylate (100 micrograms per milliliter) and chromatographing this solution as directed above. Examine the developed plate under long wavelength (366 nanometers) ultraviolet light and locate the blue fluorescent band of acridine (R_f approximately 0.5). Examination of the plate under short wavelength (254 nanometers) ultraviolet light locates the blue absorbing band of propyl quinoxaline-2-carboxylate (R_f approximately 0.5).

3. Reduce the sample zone to a fine powder by making a series of horizontal cuts with a scalpel. Gently transfer this powder with the aid of a stainless steel spatula to glassine paper; pour this material into a burette funnel atop a small glass column packed with a glass wool plug. Elute the adsorbent with ethyl acetate (about 6 milliliters), and collect the eluate to mark in a 5-milliliter volumetric flask. Examine this eluate by gas-liquid chromatography.

NOTE: Contamination of thin-layer chromatographic plates can be checked by gas-liquid chromatographic examination of an eluate prepared by processing a blank plate as in paragraph 1 above, starting at the point: ''place an edge (approximately 5 millimeters deep) of the thin-layer plate into a tray of ethyl acetate * * *.'' If the plate is contaminated, examine alternate lots of precoated thin-layer plates.

F. STANDARD CURVE

Pipette 4-milliliter aliquots of quinoxaline-2-carboxylic acid working standard solutions C, D, and E, respectively, and 4-milliliter portions of 1-propanol-sulfuric acid reagent into 50-milliliter centrifuge tubes; stopper, react, extract, and concentrate as directed in the esterification and extraction steps described in subsections C and D above; however, omit the addition of acridine to the n-hexane concentrate and do not chromatograph it by thin-layer chromatography. Instead, reconstitute the n-hexane concentrate with ethyl acetate and quantitatively transfer this solution to a 5-milliliter volumetric flask to give working standard solutions C, D, and E. The final concentrations of working standard solutions C, D, and E, are 20, 30, and 40 nanograms per milliliter, respectively, and are equivalent to 20, 30, and 40 p.p.b., respectively.

G. GAS-LIQUID CHROMATOGRAPHY

Separately inject 4 microliters of each of the working standard solutions C, D, and E (prepared as described above (F)) into the gas-liquid chromatograph to determine the retention time of propyl quinoxaline-2-carboxylate and the relative response of the EC detector. Construct a standard curve by

plotting concentration (p.p.b.) versus peak height (millimeters).

(NOTE: The reagent blank must show no interfering gas-liquid chromatographic peaks.) The peak height of propyl quinoxaline-2-carboxylate at the 30-p.p.b. level (working standard solution D) should approximate 10 percent of full-scale deflection with a retention time of 5 minutes. Follow these injections with 4-microliter injections of the tissue eluates, allowing 20 minutes between injections to clear the instrument of background peaks.

Measure the peak heights of samples and determine their concentration (p.p.b.) by reference to the standard curve.

H. CALCULATIONS

From the standard curve and the observed peak height of quinoxaline-2-carboxylic acid in the sample, determine its concentration (p.p.b.).

§ 556.110 Carbomycin.

A tolerance of zero is established for residues of carbomycin in the uncooked edible tissues of chickens.

§ 556.113 Ceftiofur.

Cattle, swine, and poultry: A tolerance for residues of ceftiofur in edible tissue is not required.

[57 FR 41862, Sept. 14, 1992]

§ 556.115 Cephapirin.

A tolerance of 0.02 parts per million (ppm) is established for residues of cephapirin in the milk and 0.1 ppm in the uncooked edible tissues of dairy cattle.

[40 FR 57454, Dec. 10, 1975]

§ 556.120 Chlorhexidine.

A tolerance of zero is established for residues of chlorhexidine in the uncooked edible tissues of calves.

§ 556.140 Chlorobutanol.

A tolerance of zero is established for residues of chlorobutanol in milk from dairy animals.

§ 556.150 Chlortetracycline.

Tolerances are established for residues of chlortetracycline in food as follows:

(a) In edible tissues and in eggs of chickens, turkeys, and ducks:

(1) 4 parts per million in uncooked kidney.

(2) 1 part per million in uncooked muscle, liver, fat, and skin.

(3) Zero in eggs.

(b) In edible tissues of swine:

(1) 4 parts per million in uncooked kidney.

(2) 2 parts per million in uncooked liver.

(3) 1 part per million in uncooked muscle.

(4) 0.2 part per million in uncooked fat.

(c) In edible tissues of calves:

(1) 4 parts per million in uncooked liver and kidney.

(2) 1 part per million in uncooked muscle and fat.

(d) In edible tissues of beef cattle and nonlactating dairy cows:

(1) 0.1 part per million in uncooked kidney, liver, and muscle.

(2) Zero in uncooked fat.

(e) Zero in milk.

(f) In edible tissues of sheep:

(1) 1 part per million in uncooked kidney.

(2) 0.5 part per million in uncooked liver.

(3) 0.1 part per million in uncooked muscle.

[40 FR 13942, Mar. 27, 1975, as amended at 49 FR 22634, May 31, 1984]

§ 556.160 Clopidol.

Tolerances for residues of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in food are established as follows:

(a) In cereal grains, vegetables, and fruits: 0.2 part per million.

(b) In chickens and turkeys:

(1) 15 parts per million in uncooked liver and kidney.

(2) 5 parts per million in uncooked muscle.

(c) In cattle, sheep, and goats:

(1) 3 parts per million in uncooked kidney.

(2) 1.5 parts per million in uncooked liver.

(3) 0.2 part per million in uncooked muscle.

(d) In swine: 0.2 part per million in uncooked edible tissues.

(e) In milk: 0.02 part per million (negligible residue).

§ 556.163 Clorsulon.

Tolerances are established for residues of clorsulon in cattle as follows: